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Multivariate Curve Resolution of *p*-Coumaric Acid *o*-Hydroxylation Reaction Mechanism Catalyzed by Tyrosinase

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Multivariate curve resolution is able to extract the number of components involved in a complex spectral feature and attribute the resulting spectra to chemical compounds. The quantification of the individual spectral contributions develop kinetic models for the process with or without a prior knowledge. Data elaboration by multivariate curve resolution was able to explain the kinetics of degradation, giving the pure spectra of the involved species and the respective concentration variation. In this study, *p*-coumaric acid *ortho* hydroxylation of mushroom tyrosinase investigated using UV-VIS absorption spectra and chemometric resolution techniques. From the spectra of the reaction between 200 and 600 nm, number of components and their mole fractions in the reaction solution were determined using chemometric resolution technique. After the determination of components, multivariate curve resolution-alternating least square by initial estimates of spectral profiles and proper constraints, was used to resolve the data matrix into pure concentration and spectral profiles. Using chemical rank determination and subspace comparison the chemical species or chemical rank of matrix obtained three. So, there are three species during monophenolase reaction of mushroom tyrosinase.

Key Words: Multivariate curve resolution-alternating least square, Mushroom tyrosinase, Monophenolase.

INTRODUCTION

Tyrosinase or polyphenol oxidase is a copper enzyme widely distributed throughout the phylogenetic scale. It catalyses the hydroxylation of monophenol to *o*-diphenols and their subsequent oxidation to *o*-quinones, in both cases by molecular oxygen^{1,2}. The kinetic behaviour of tyrosinase is very complex due to the contemporaneous occurrence of the enzymatic oxidation of monophenol and *o*-diphenol to *o*-quinone, on the one hand and the coupled non-enzymatic reactions of *o*-quinone, on the other^{3,4}.

Chemometric methods like principal component analysis and partial least squares regression have been used successfully in many applications over the years. The technique is well established in many analytical fields, like UV/VIS, NIR, IR spectroscopy⁵ gas and liquid chromatography⁶ and manufacturing processes⁷.

Much research has been performed on solving the mixture analysis problem and extracting real spectra and concentration profiles from overlapping spectral data without making any prior assumptions about the composition of the system. Several mixture analysis methods are known, like evolving factor analysis⁸, fixed size moving window evolving factor analysis⁹,

target factor analysis¹⁰, classical curve resolution¹¹, weighted curve resolution¹², multivariate curve resolution¹³ and to a certain extent also techniques like parallel factor analysis¹⁴. Using these techniques, we can mathematically estimate the evolution of the chemical contributions over time for a specific experiment. The measured spectral information at different wavelengths reveals the specific absorption and also the morphologies of the compounds due to the scatter produced when particles or solid biomass is present. However, the resulting spectra are difficult to interpret and they lack specificity. This disadvantage can be solved using curve resolution methods using the following three objectives of this study. The first is resolve the number of chemical compounds and intermediates simultaneously present in the mixture from a complex spectral signature. The second identification of these species by transforming mathematical solutions to real spectra and increasing their specificity. The third to quantify each component and transfer this information to a kinetic model without any prior assumption or knowledge of the chemical model involved. Thus, we demonstrate the possibilities and power of this method to estimate and quantification of intermediates during the cresolase reaction that mushroom tyrosinase